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RESTRICTION IN THE REPERTOIRE OF DETECTABLE AUTOANTIBODIES IN POLYCLONAL B CELL ACTIVATIONS AND THE MIMICRY OF AUTOANTIGENS BY IDIOTOPES

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Despite the existence of erythrocyte-autoreactive B cells in normal animals, erythrocyte-autoantibodies could not be detected during polyclonal B-cell activation (PBA) both in patients with visceral leishmaniasis and in bacterial lipopolysacharide (LPS) – injected mice. The failure to detect these autoantibodies in mice with PBA did not seem to be due to suppressor-cell activity, since (1) transfer of spleen cells from LPS-treated mice to naive recipients did not affect the erythrocyte-autoantibody response elicited by subsequent injections of rat erythrocytes and (2) low doses of X-radiation did no lead to erythrocyte-autoantibody detection in LPS-treated mice. The possibility that the detection of erytrocyte-autoantibodies could be affected by autoantibodies with idiotopes mimicring erythrocyte epitopes, the synthesis of which would also be triggerred in PBA, is discussed. Indirect evidence for the existence in normal animals of an expanded lymphocyte population with DNP-binding, Ia-mimicring antigen receptors is presented.

The existence in normal animals of B lymphocytes recognizing autoantigens such as IgG, heart tissue, thyroglobulin, DNA or erythrocytes has been established by the direct demonstration of autoantigen-binding cells (Unanue, 1971; Bankhurst, et al., 1973; Bankhurst & Williams, 1975) or inferred from the detection of autoantibodies in normal animals or human subjects sensitized with cross-reactive antigens (Kaplan & Meyeserian, 1962; Playfair & Marshall-Clarke, 1973), depleted of a lymphocyte sub-population (Cantor & Gershon, 1979), subjected to adult thymectomy followed by low doses of X-radiation (Penhale et al., 1973) or affected by polyclonal B-cell activation (PBA; Rosenberg, 1978; Louis & Lambert, 1979; Galvão-Castro et al., 1984). Interestingly, in this last case the repertoire of detected autoantibodies does not correspond completely to the specificities of existing B lymphocytes. Thus, thyroglobulin-autoantibodies have not been detected in mice injected with the polyclonal B cell activator bacterial lipopolysaccharyde (LPS-Esquivel et al., 1977) or during the PBA of murine (Daniel-Ribeiro et al., 1982) or human (Daniel-Ribeiro et al., 1983) malaria. A similar situation was described for erythrocyte-autoantibodies, which could not be demonstrated in human malaria (Facer, 1980). This can not be ascribed to a proposed role for antigen-mediated stimulation in PBA (Watson et al., 1973; Daniel-Ribeiro et al., 1982, 1983), since erythrocyte-autoantigens are certainly available in large amounts to the immune system.

Two additional hypotheses at least can be adduced to account for the discrepancies between the repertoire of detectable autoantibodies and of autoreactive B cells pointed out above. First, since it has been shown that the T-cell compartment can modulate PBA (Smith et al., 1983), it could well hapen that some autoreactive B lymphocytes would be under more stringent T-lymphocyte control than other lymphocytes and therefore would not be stimulated during PBA. Second, the undetectable antibody specificities could be inside what has been called "high connectivity" segments of the idiotype (Id) network by Holmberg & Coutinho (1985). "High connectivity" lymphocytes would be under strong, mutually controlling influences, and would be unable to undergo clonal expansion upon exogenous stimulation (Holmberg & Coutinho, 1985). Some aspects of these two hypotheses, which are obviously not mutually exclusive, are dealt with below.

NO FORMATION OF ERYTHROCYTE-AUTOANTIBODIES IN VISCERAL LEISHMANIASIS AND IN LPS-INDUCED PBA

Patients with visceral leishmaniasis develop an intense PBA with hypergammaglobulinaemia, formation of autoantibodies against DNA and IgG (Galvão-Castro et al., 1984) and erythrocyte-bound IgG (Pontes de Carvalho et al., 1986).

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These erythrocyte-bound IgG, however, are not erythrocyte-autoantibodies since they, after elution from the erythrocytes, did not bind back to erythrocytes bearing all known autoimmunogenic blood group antigens (Fig. 1). In keeping with this finding, mice which were weekly injected i.p. with LPS for up to 5 months did not develop detectable erythrocyte-autoantibodies (Fig. 2a), not with standing the induction of a well characterized PBA, with hypergammaglobulinaemia and with formation of DNA- and IgG-autoantibodies.

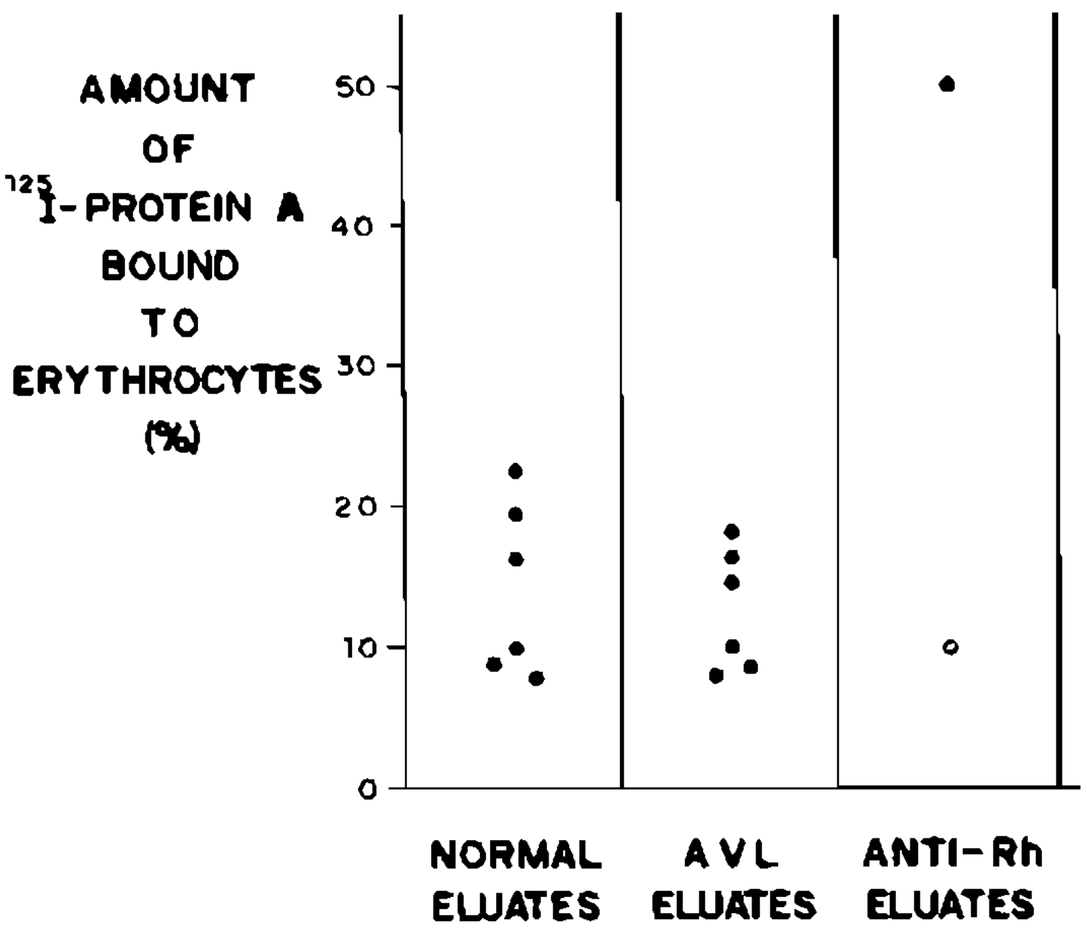

Fig. 1: No sensitization of normal erythrocytes with eluates prepared with ether from the erythrocytes of patients with American visceral leishmaniasis (AVL eluates). Fifty microlitre volumes of packed erythrocytes bearing all major autoantigenic blood groups (●), or of packed Rh-negative erythrocytes (o), were incubated with 1-2 ml of eluates and then tested in a protein A binding immunoradiometric assay. Circles correspond to the amounts of radiolabelled protein A bound to erythrocytes pre-incubated with individual eluates (from Pontes de Carvalho et al., 1986).

FAILURE TO DETECT SUPPRESSOR CELL ACTIVITY AFFECTING ERYTHROCYTE-AUTOANTIBODY FORMATION IN PBA

Spleen cells from LPS-treated A/Sn or CBA mice, when transferred to syngeneic recipients, did not affect the subsequent elicitation of erythrocyte-autoantibodies by injections of cross-reactive rat erythrocytes (Fig. 2b). These findings would indicate that suppressor cells, which would be activated during PBA, would not play a role in the control of erythrocyte-specific autoantibody responses. This view was reinforced by the finding that Swiss outbred mice subjected to sublethal doses of X radiation (which is known to preferentially affect the suppressor cell compartment in different animal species; Gengozian & Makinodan, 1958; Dixon & McConahey, 1963; Taliaferro & Taliaferro, 1969; Tada et al., 1971) did not respond to LPS with the formation of erythrocyte-autoantibodies (Table). Therefore, the absence of detectable erythrocyte-autoantibodies in PBA does not seem to be due to suppressor cell activity.

POSSIBLE ROLE FOR IDIOTOPES MIRRORING AUTOANTIGENS IN THE CONTROL OF AUTOIMMUNITY

The existence in normal animals of idiotopes mimicring other self-structures, such as Ia, has been recently demonstrated (Sim & Augustin, 1983; Holmberg et al., 1984), and it can be easily visualized how this mimicry of self-structures, either by suppressor T cells receptors or suppressive antibodies, could be of importance in inhibiting autoimmune responses. In fact, the existence of clonally expanded B cells bearing mirror images of other autoantigens (para-internal images) could function as a first line of defense against autoantibodies formed during polyclonal B cell activations. The polyclonal B cell activator would induce the synthesis of both the autoantibody and of its complementary, blocking or inhibitory anti-autoantibody. Indeed, idiotype – anti-idiotype immune complexes have been demonstrated in LPS-treated mice (Rose et al., 1982).

The clonal expansion of para-internal image-bearing lymphocytes could be favoured by the clonal abortion or clonal inactivation of anti-para-internal image lymphocytes, i. e., of autoreactive lymphocytes (by soluble autoantigen, for instance). A similar hypothesis has been proposed to explain why the injection of an exogenous antigen may lead first to the formation of "anti-Id" and secondly of "Id" (Id would be blocked by the antigen, and the anti-Id-producing lymphocytes would be released from Id-mediated control; Cerny & Kelsone, 1984).

In fact, these "Id" released from "anti-Id" control (because anti-Id is an autoantibody and therefore subjected to vigorous suppression) may well correspond to some of the structures that have been called cross-reactive or recurrent idiotypes (Kard & Noble, 1978; Reth et al., 1979; Metzger, et al., 1980; Bona et al., 1980; Capra et al., 1982; Kennedy & Dreesman, 1983; Cooke et al., 1983; Zanetti et al., 1983; Cunningham-Rundle & Cheung, 1985). It would certainly be of interest to determine the relative sizes of para-internal image and of anti-para-internal image (autoreactive) clones,

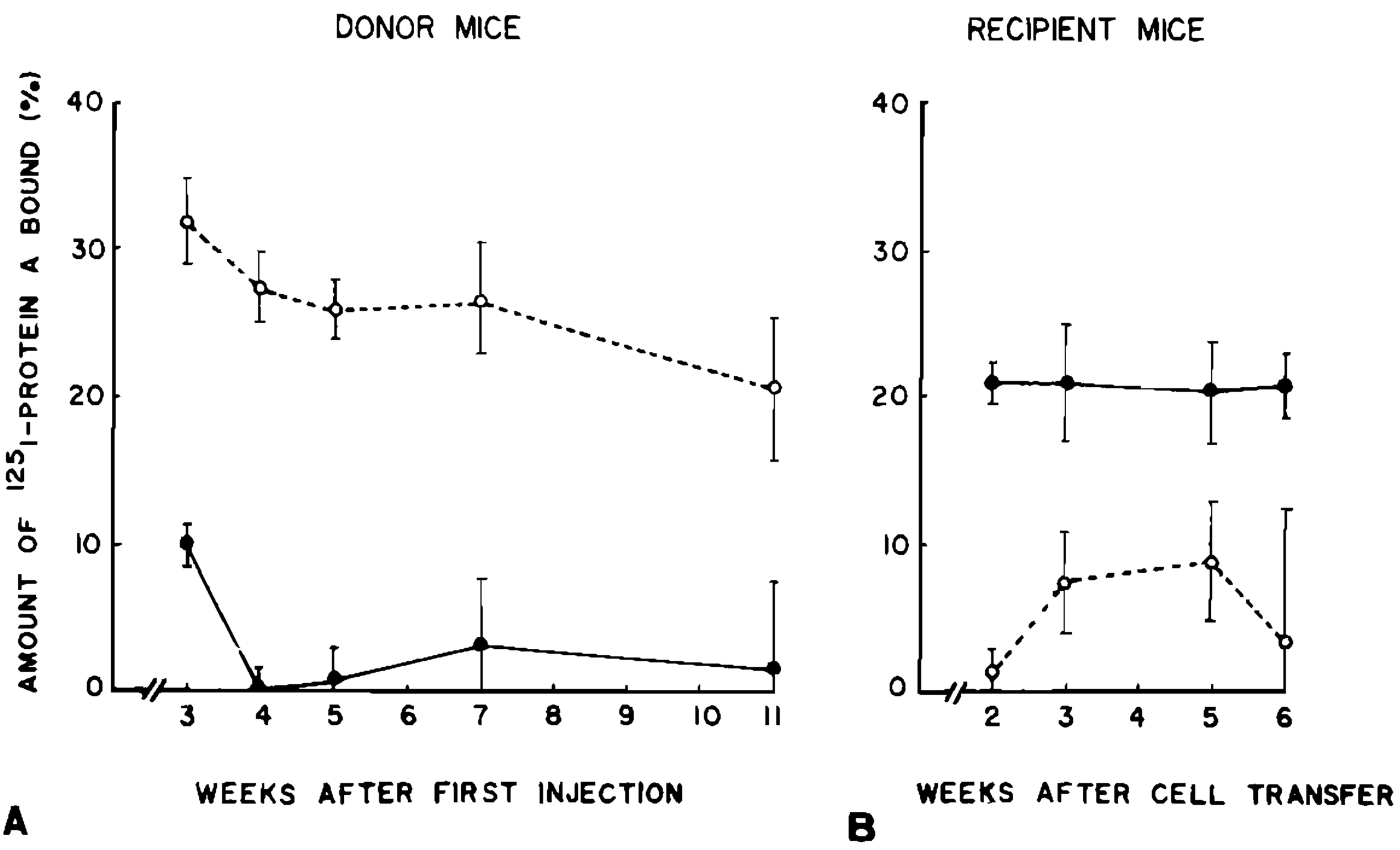

Fig. 2: Amount of erythrocyte-bound immunoglobulin in CBA mice receiving: (1) ten weekly i.p. injections of 20 µg LPS (●—●, left); (2) four weekly i.p. injections of 2×10^8 rat erythrocytes (○—○, left); (3) one i.v. injection of 7×10^7 spleen cells from the LPS-treated mice referred to in item *1* above, followed one day later by 4 weekly i.p. injections of 2×10^8 rat erythrocytes (●—●, right); (4) one i.v. injection of 7×10^7 spleen cells from the rat erythrocyte-sensitized mice referred to in item *2* above, followed one day later by 4 weekly i.p. injections of 2×10^8 rat erythrocytes (○—○, right).

Amounts of erythrocyte-bound immunoglobulin were estimated by incubating the washed erythrocytes with rabbit auto-mouse immunoglobulin followed by radiolabelled protein A (modified from Pontes de Carvalho et al., 1985). Vertical bars represent standard deviations of means obtained from six mice.

TABLE

No detection of erythrocyte-autoantibodies in LPS-treated outbred Swiss mice previously subjected to 400 rad X-irradiation

Treatment *	Incidence of hypergammaglobulinaemia	incidence of immunoglobulin-coated erythrocytes **
X ray	5/7 +	0/7 +
X ray + LPS	7/7	0/7
LPS	4/4	0/4

* Mice were subjected to X-irradiation on day -1 and injected with 20, 25 and 50 µg of LPS on days 0, 10 and 17, respectively.

** As assessed by the direct Coombs' test.

\+ No. of animals with hypergammaglobulinaemia or with immunoglobulin-coated erythrocytes (determined on days 5, 15 and 22) / total no. of animals in group.

to see whether the former are disproportionaly larger (clonally expanded) than the latter, as suggested above.

DNP- or TNP-specific B cells are unexpectedly well represented within the total B-cell repertoire (Eisen et al., 1968; Yamada & Yamada, 1969; Watson et al., 1973; Cambier & Corley, 1981; Daniel-Ribeiro, 1983) and, in accordance with the hypothesis presented above, one would explain this fact by postulating that the binding sites for DNP (TNP) on lymphocytes would be mirror images of an autoantigen. The lymphocytes reacting with this binding site (i.e., having anti-anti-DNP/TNP specificity), which would be at least partially responsible for controlling the anti-DNP (TNP) responses, would be autoreactive and therefore would be either missing or operationally suppressed, The anecdotal report of one monoclonal antibody, reacting with and anti-Ia mon-

oclonal antibody (possibly with idiotope, or idiotopes, mimicking Ia), having anti-TNP activity (Holmberg et al., 1984) would be suggestive that the autoantigen mirrored by TNP-binding sites, or by cross-reactive (Little et al., 1969) DNP-binding sites, is Ia. If this assumption were correct, one would predict that TNP and/or DNP would be an *external* image of anti-Ia paratopes (Fig. 3) and, therefore, should bind to Ia. Indeed, fluorescein-labelled DNP-bovine serum albumine, when incubated with cryostat

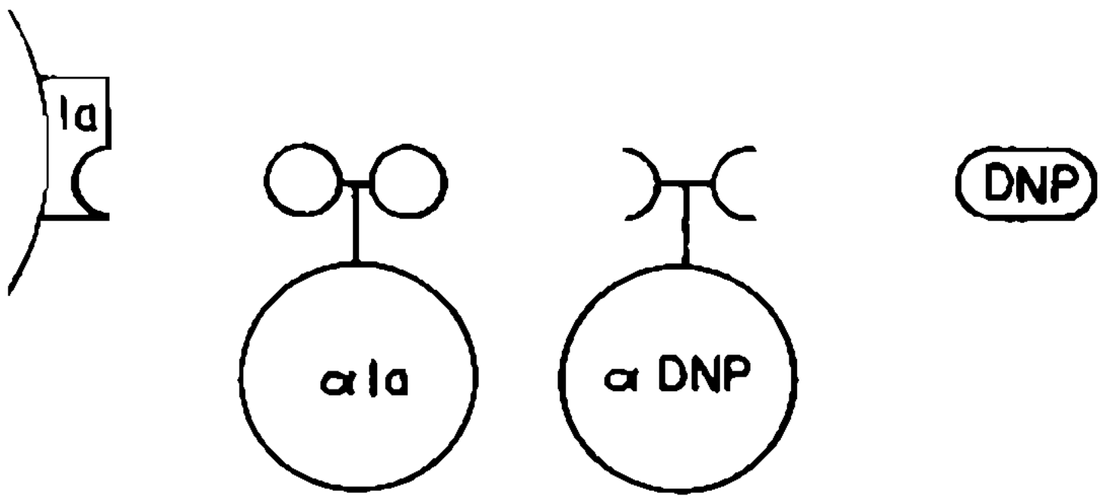

Fig. 3: Mimicry of anti-Ia (αIa) by DNP. The anti-DNP (αDNP) idiotope or paratope represented in this figure is an *internal* image of Ia (DNP in its turn is an *external* image of Ia (DNP in its turn is an *external* image of αIa). Lymphocytes with this (αDNP) specificity are present in relatively large numbers in normal animals (Eisen et al., 1968; Yamada & Yamada, 1969; Watson et al., 1973; Daniel-Ribeiro, 1983) and would play a role in keeping an autoimmune response to Ia under control.

sections of mouse thymus, was found to bind to Ia-rich areas. Moreover, the specificity of the binding could be ascertained by the demonstration that previous incubation of the thymic sections with an anti-Ia serum was able to completely block the subsequent binding of DNP Whether this expanded population of lymphocytes with (DNP-binding) internal images of Ia would play a role in controlling an Ia-reactive response during PBA is an attractive possibility that deserves further investigation.

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